ARL-TDR-63-17

CHARACTERIZATION OF HORMONAL STEROIDS OF THE CHIMPANZEE

Enrico Forchielli, Ph.D.
Fred Elmadjian, Ph. D.

TECHNICAL DOCUMENTARY REPORT NO. ARL-TDR-63-17

May 1963

6571st Aeromedical Research Laboratory
Aerospace Medical Division
Air Force Systems Command
Holloman Air Force Base, New Mexico

Project 6892, Task 68921

(Prepared under Contract No. AF 29(600)-2439 by the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts.)

FOREWORD

The authors acknowledge the invaluable participation of Captain Erwin R. Archibald, Captain William E. Ward, and Captain Thomas L. Gleason, III, who were project officers during the course of these studies.

Appreciation is also expressed to Captain (Dr.) Jerry Fineg, who as Chief of Vivarium, directed blood sampling and urine collections, and Major (Dr.) Robert H. Edwards for his professional advice.

Recognition of the technical assistance of the following personnel is also in order: MSgt Edward C. Dittmer, AlC Charles H. S. Watkins, and AlC George V. Pegram.

The authors are deeply indebted to colleagues at the Worcester Foundation for Experimental Biology, particularly Dr. Constantin Gherondache, Mr. Edwin T. Lamson, and Mr. Norman Gibree for the technical aspects relating to the determinations reported in this document.

Finally, appreciation is expressed for the valuable advice and support of Dr. Gregory Pincus and Dr. Ralph I. Dorfman.

ABSTRACT

This report attempts to establish (a) the qualitative and quantitative nature of steroid hormones of the chimpanzee, and (b) to report changes observed in adrenal cortical functions during simulated and actual space flights. Steroid determinations made include: Blood: plasma 17-hydroxycorticosteroids (17-OHCS) and cholesterol; Urine: 17-OHCS, 17-ketosteroids (17-KS), estrone, estradiol, estriol, pregnanediol, pregnanetriol, and aldosterone. Quantitative chromatography of 17-KS was also accomplished. Analyses were made on both the mature and immature chimpanzee.

PUBLICATION REVIEW

This technical documentary report has been reviewed and is approved.

HAMILTON H. BLACKSHEAR
Lt Colonel, USAF, MC
Commander,
6571st Aeromedical Research Laboratory

CARE AND HANDLING OF THE SUBJECTS

The animals used in this study were handled in accordance with the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

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I. INTRODUCTION AND PURPOSE

The choice of the chimpanzee as a species of primate for studying biological aspects of space flight was prompted by its capabilities in psychomotor testing and its relatively small size. The animals studied were those participating in the Project Mercury program and were located at the Air Force Missile Development Center, Holloman Air Force Base, New Mexico. Characterization of steroid hormones of the chimpanzee was desirable for purposes of evaluating the hormonal adjustment in space flight. The results obtained would be of value to predict the possible changes that might occur in human space flights. This is a preliminary report attempting to establish (a) qualitative and quantitative nature of steroid hormones of this species, and (b), to report changes observed in adrenal functions during simulated and actual space flights. Steroid determinations made include: Blood: plasma 17-hydroxycorticosteroids (17-OHCS) (Ref. 1) and cholesterol (Ref. 2); Urine (17-OHCS) (Ref. 3), 17-ketosteroids (17-KS)(Ref. 4); Estrone, estradiol, estriol (Ref. 5), pregnanediol, pregnanetriol (Ref. 6), and aldosterone (Ref. 7). Quantitative chromatography of 17-KS was also accomplished (Ref. 8). Analyses were made on both the mature and immature chimpanzee.

II. EXPERIMENTAL DESIGN

The immature chimpanzee was subjected to stresses involving (a) temperature humidity test (THI): sitting 20 hours at temperatures of 70 and 80°F ("the thermally neutral"); 85, 90, 95, and 100°F ("thermally stressful") with 50 percent humidity, without food and water; (b) acceleration and deceleration tests (AD) simulating launch and re-entry conditions, and (c), actual suborbital flight of chimpanzee No. 65 (HAM) (MR-2)*.

Each experiment was carried out over a 5-day period, with blood sample No. 1 collected on the morning of the first day (control), sample No. 2 collected immediately after termination of the stress, which took place on the second day, and sample No. 3 collected 48 hours after termination of the stress test. Urine collections were scheduled so that there were two collections for each day: the first sample from 0730 to 1600 (day) and the second sample from 1600 to 0730 (night) the following day, and so on for a total of ten samples.

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*Mercury Redstone 2

III. CHARACTERIZATION OF STEROIDS

A. 17-Hydroxycorticosteroids (17-OHCS) and 17-Ketosteroids (17-KS)

Table I presents the data of the control day which was divided into day (collection 0730-1600) and night (1600-0730) samples. It will be noted that the immature chimpanzee indicates the possibility of a diurnal variation in both 17-OHCS and 17-KS excretion. The mean daily excretion of 17-OHCS of immature chimpanzees is 2.8 mg. while that of the adult chimpanzee is 3.8 mg. The 17-KS excretion of 4.0 mg. per day for the immature chimpanzee is somewhat higher than the 17-OHCS excretion.

TABLE I

17-HYDROXYCORTICOSTEROIDS (17-OHCS) and 17-KETOSTEROIDS (17-KS) EXCRETION OF IMMATURE CHIMPANZEE ON CONTROL DAY*

	DAY (0730-1600)	NIGHT (1600-0730)
17-OHCS	.16 ± .028 (20)	.10 ± .017 (23)
17-KS	.23 ± .030 (9)	.13 ± .013 (12)

*mg/20 mg. creatinine

There are two factors which should be mentioned in evaluating the above data. Chimpanzee urine, when processed for 17-OHCS determinations, frequently gave a high pink blank value. Approximately 20 percent of the urine samples analyzed gave such high blanks that no value could be obtained. It is highly possible that the tendency for high blanks reduced the titer in a large proportion of determinations obtained. As for the 17-KS, it was discovered that only 33 to 63 percent of the total Zimmerman titer was recovered quantitatively after chromatography procedure.

B. Chromatography of 17-KS

Preliminary results of chromatography analysis of the urine of immature chimpanzees indicated the presence of 11ß-hydroxyetiocholanolone, 11ß-hydroxyandrosterone, dehydroepiandrosterone and traces of etiocholanolone and androsterone. Quantitative estimations were not feasible because of the small amounts. The quantitative results of three daily collections obtained from adult chimpanzee No. 3 (VICKIE) are presented in Table II. During the course of the collection there was some question of whether chimpanzee No. 3 (VICKIE) was pregnant; however, on 3 April 1961, menstruation was recorded for this chimpanzee.

All samples were hydrolyzed enzymatically with ß-glucuronidase and chemically using the solvolysis method (Ref. 9). Individual 17-KS were analyzed after paper chromatographic separation. It should be noted that only 33 to 63 percent of the total 17-KS was recoverable quantitatively. Normal human urine so processed in our laboratories indicate 80 to 90 percent recoverable material. It appears from these data that significant amounts of non-specific chromogens are measurable in the total 17-KS urine of this adult female chimpanzee. These results permit us to consider that the 17-KS excretion of the mature female chimpanzee parallels qualitatively and quantitatively the data observed for the human (Ref. 10, 11, 12, 13, 14).

C. Aldosterone Excretion

Twenty-four hour urine samples are necessary for aldosterone determination. The values for the immature chimpanzee, ranging from 0.00 to 2.2 mcg. per day are presented in Table III. We have had results indicating that the immature chimpanzee is capable of excreting as high as 27.7 mcg. per day under the stress of 90°F and the deprivation of both food and water.

D. Plasma 17-Hydroxycorticosteroids

All the data available for plasma 17-OHCS on the immature chimpanzee are depicted in the figure on page 6. It will be noted that the values for blood samples No. 1 (pre-stress) and No. 3 (post-stress) show similar distributions indicating that the "basal"17-OHCS ranges from 10 to 20 mcg. percent. Normal values for human subjects as well as for the Rhesus monkey are within this range.

TABLE II

17-KETOSTEROIDS - CHIMPANZEE NO. 3 (VICKIE)

mcg/1.0 g. creatinine

Total 17-KS	4430	8230	3600
Chromatography of 17-KS			
11-OH-E	222	355	240
11-OH-A	139	139	100
11-K-E	612	854	948
11-K-A	185	299	73
DHEA	408	512	483
Etio	231	270	204
Andro	174	244	225
Pre-andro	78	40	48
Polar Res.	26	46	256

Abbreviations:

11-OH-E:	11β-Hydroxyetiocholanolone
11-OH-A:	11β-Hydroxyandrosterone
11-K-E:	11-Ketoetiocholanolone
11-K-A:	11-Ketoandrosterone
DHEA:	Dehydroepiandrosterone
Etio:	Etiocholanolone
Andro:	Androsterone
Pre-andro:	Any material on chromatogram less polar than androsterone.
Polar Res:	Chromogenic material remaining at the origin of paper chromatograms.

TABLE III

ALDOSTERONE EXCRETION IN
IMMATURE FEMALE CHIMPANZEE

Chimpanzee No.	Total Creatinine	Aldosterone mcg/0.5 g Creat.**
35	372	0.60
41*	421	23.70
46	500	1.00
46	423	1.20
46	465	2.20
49	645	0.00
49*	813	27.70
50	692	1.50

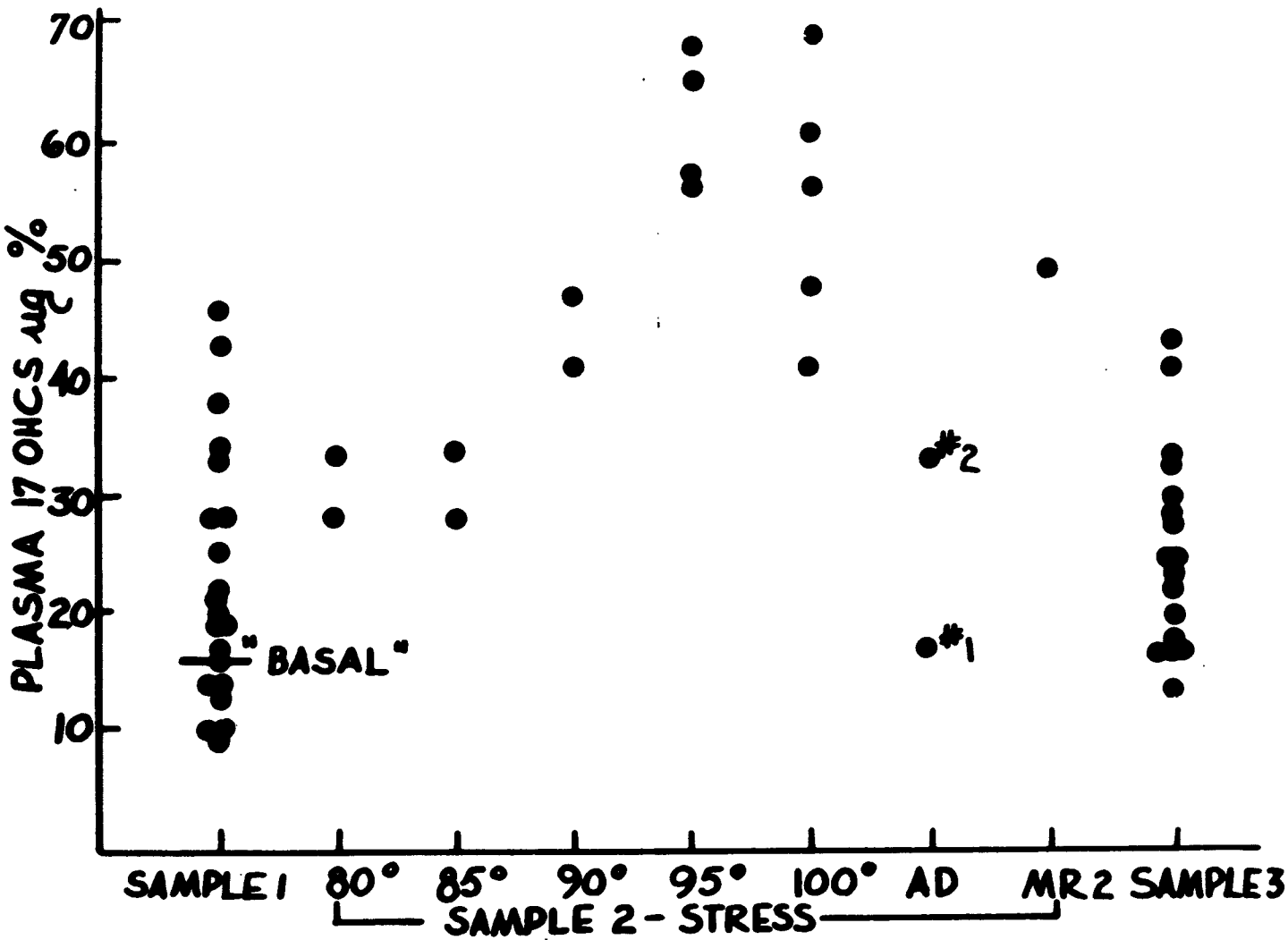
* After Stress
** Equivalent to daily excretion

TABLE IV

PREGNANEDIOL AND PREGNANETRIOL EXCRETION
OF IMMATURE CHIMPANZEE*

	N	Mean
Pregnanediol	23	.23 (.04-.60)
Pregnanetriol	22	.40 (.18-.83)

*mg/day



PLASMA 17-HYDROXYCORTICOSTEROIDS (17-OHCS)

E. Pregnanediol and Pregnanetriol Excretion

The pregnanediol and pregnanetriol values for the immature chimpanzee are presented in Table IV. We wish to point out the range of values depicted in the table, especially the levels of low concentration.

Data were also obtained on the urinary excretion of pregnanediol and pregnanetriol of three adult female chimpanzees (Table V). Chimpanzee No. 132 (FITA) was sampled on 26 June 1961. According to the records, this sample was obtained during post-ovulatory phase of the menstrual cycle. Chimpanzee No. 135 (BETA) was sampled on 23 May 1961. This sample appears to have been obtained in mid-cycle. Chimpanzee No. 3 (VICKIE) was sampled on 28 March 1961 and on 31 March 1961. Chimpanzee No. 3 (VICKIE) was suspected of being pregnant during the period of the sampling. The fact that the animal did flow on 3 April 1961 does not rule out this possibility. The pregnanediol data supports the view that chimpanzee No. 3 (VICKIE) may have been pregnant; the data on the estrogens to be presented in the next section also supports this view. The relatively low values for both pregnanediol and pregnanetriol for chimpanzee No. 135 (BETA) is consistent with values expected at mid-cycle. The value of 1.21 mg. obtained indicates that chimpanzee No. 132 (FITA) was sampled during the luteal phase of the cycle.

F. Estrogens

The urine obtained from an immature male chimpanzee was hydrolyzed successively by glucuronidase and the solvolysis method for urinary steroid sulfates, was extracted and chromatographed for separation of estrone (E_1), estradiol (E_2) and estriol (E_3). Quantitative estimation of the separated estrogens was made fluorometrically. The results (Table VI) indicate that the urine of the immature male chimpanzee contains E_1, E_2, and E_3 principally as a glucuronide and sulfate. E_1 appears principlally as a sulfate, while E_2 and E_3 are mainly present in the urine as glucuronides. We do not have data from pre-adolescent humans for comparative purposes. We are aware that the values presented in Table VI are much higher than those reported in the literature by other methods.

Estrogen analysis was performed on three samples obtained from chimpanzee No. 3 (VICKIE) (see above) on the following dates: 27 March, 28 March, and 31 March 1961. The data for total estrogens are presented in Table VII.

TABLE V

PREGNANEDIOL AND PREGNANETRIOL ON MATURE FEMALE CHIMPANZEE

Chimpanzee	Age Years	Weight Pounds	Cycles Days	Initiation of last flow	Date of sample	Pregnanediol mg/24 hrs.	Pregnanetriol mg/24 hrs.
Fita	15	95	40-50	5-15-61	6-26-61	1.21	2.80
Beta	8	88	60	4-15-61	5-23-61	.30	.62
Vickie*	15	96	Pregnant?		3-28-61	7.2	---

*Flow on 4-3-61

TABLE VI

EXCRETION OF ESTROGENS
IN IMMATURE MALE CHIMPANZEE (NO. 42)

mcg/ .5 GM. creatinine*

	ß-Gluc.**	SO_4***	Total	% of Total ($E_1 + E_2 + E_3$)
10 May 1960				
Estrone (E_1)	.90	1.26	2.16	37.7
Estradiol (E_2)	.73	.48	1.21	22.2
Estriol (E_3)	1.42	.65	2.07	38.1
11 May 1960				
Estrone	.75	.86	1.61	41.1
Estradiol	.56	.41	.97	24.7
Estriol	.95	.39	1.34	34.2

*Equivalent to daily excretion

**ß-Glucuronidase hydrolysis: includes free (trace amounts) plus glucuronides

***Sulfates in addition to values under**

It may be noted that the estrogen values, especially for estriol, are surprisingly high. Published data (Ref. 5) give the following values for the three estrogens in early pregnancy urine: E_1, 3.5 - 13.5 mcg/L; E_2, 1.6 - 6.2 mcg/L; and E_3, 60.6 - 194.4 mg/L. It is of interest that the data presented on chimpanzee No. 3 (VICKIE) compares favorably with the published results. The data on pregnanediol as well as estrogens support the possibility that chimpanzee No. 3 (VICKIE) was pregnant at the time of the sampling, even though flow was recorded on 3 April 1961.

TABLE VII

ESTROGEN EXCRETION OF
ADULT FEMALE CHIMPANZEE "VICKIE"

Total Estrogens

mcg/1.0 g. creatinine

	27 March 1961	28 March 1961	31 March 1961
Estrone	0.185	0.285	3.60
Estradiol	3.90	8.25	7.7
Estriol	133.5	160.5	30.5

IV. ADRENAL CORTICAL CHANGES DURING SIMULATED AND ACTUAL SPACE FLIGHTS

A. 17-Hydroxycorticosteroids (17-OHCS) and 17-Ketosteroids (17-KS)

In Table VIII are presented the 17-KS results, indicating varying degrees of increases in this index during the stress (samples No. 3 and 4). Of particular interest are samples No. 4 of THT-17 (animal No. 42) and THT-18 (animal No. 35). The diurnal variation of 17-KS may be observed in the data. In Table IX are depicted the 17-OHCS data. Here again, we wish to draw attention to the increased 17-OHCS excretion, especially in THT 11 and 17. The urine data available from chimpanzee No. 65 (HAM) (MR-2) during the suborbital flight, indicate moderate to marked increases in 17-OHCS. The increases in both 17-OHCS and 17-KS observed during the suborbital and orbital flight were in the same order as those observed in the severe temperature humidity test stresses.

TABLE VIII

URINARY 17-KETOSTEROIDS

mg/20 mg. creatinine

TEST	ANIMAL NO.	TEMP. F	1	2	3	4	5	6	7	8	9	10
THT 16	41	80	--	.15	--	.16	.23	.23	.46	.11	.32	--
THT 16	49	80	.23	.11	.10	---	---	.31	.14	.11	.15	.09
THT 17	42	90	---	.09	.11	.52	.09	.29	.16	---	.31	.10
THT 18	35	90	.22	.15	---	.41	---	.40	.36	.32	.23	.10
THT 18	50	90	.31	.20	.23	---	.41	.68	.26	---	.31	.23
AD-1	42		---	.16	.19	.23	.28	.11	---	.15	.22	.22
MR-2	65		.11	.07	.11	---	---	.09	.20	.08	.08	.08

TABLE IX

URINARY 17-HYDROXYCORTICOSTEROIDS

mg/20 mg creatinine

TEST	ANIMAL NO.	TEMP. F	1	2	3	4	5	6	7	8	9	10
THT 10	42	80	.17	.12	.22	---	.15	.14	.04	.13	.06	.07
THT 13	42	80	.08	.07	.14	.17	.24	.13	.06	.06	.07	.17
THT 11	42	85	.13	.09	.31	.20	.08	.15	.32	.07	.08	.10
THT 17	42	90	---	.03	.20	.82	.12	.11	---	.24	---	---
AD-1	42		.16	.10	.07	---	---	---	.03	.04	.11	.08
MR-2	65		---	.03	---	.20	.06	---	.08	.02	---	.04

B. Plasma Cholesterol and 17-Hydroxycorticosteroids

The plasma cholesterol data are presented in Table X. Increases in plasma cholesterol are observed in both "thermally neutral" and "thermally stressful" conditions. No correlation is evident between the increases in plasma cholesterol and the increasing stress experienced by the chimpanzee in terms of temperature. It is significant to observe that where the experiments were terminated before the full programmed 20-hour period, no consistent increases were observed in this index. We wish to emphasize that plasma cholesterol is not being presented as an index of adrenal cortical function. The data are of interest only in the finding that this measure showed increases in both "thermally neutral" and "thermally stressful" conditions when the experiments were run through the full 20-hour period.

TABLE X

PLASMA CHOLESTEROL

mg. %

THT	Animal No.	Temp. F	Pre-	Stress	Post-
4	46	80	271	297	281
5	35	80	196	233	196
8	41	80	218	354	324
10	42	80	165	226	184
13	42	80	156	208	196
11	42	85	133	201	145
12	44	85	208	199	173
14	35	85	242	304	234
18	50	90	253	285	280
18	35	90	242	296	248
19	41	95	219	344	292
23	41	100	269	340	381
23	50	100	280	279	---
24	35	100	273	306	269
24	46	100	259	250	249
25	49	100	292	342	252
25	50	100	263	250	243

The plasma 17-OHCS data are presented in the figure on page 6. As mentioned previously, the "basal" 17-OHCS value of an immature chimpanzee may be considered to be approximately 16 mcg percent. Quantitative increases in sample No. 2 (stress sample) of the 17-OHCS data were observed with increases in temperature. The ceiling appears to be approximately 70 mcg percent. It is noted that AD-1 shows less of an increase than AD-2. Furthermore, the result obtained on chimpanzee No. 65 (HAM) (MR-2) immediately after the suborbital flight, was somewhat less than the value obtained in the 20-hour stress period under 95°F conditions. Interpretation of the above data is difficult due to the varying degrees of time lapse from the point of termination of the stress to the time of sampling.

V. CONCLUSIONS

Preliminary data characterizing steroids of the chimpanzee indicate similarity both qualitatively and quantitatively with those found in the human.

VI. RECOMMENDATIONS

These studies on steroidology of the chimpanzee should be continued with the modern technics now available. This can be accomplished by using the double isotope method (H^3 and C^{14}), which will give accurate secretion rates of steroid hormones such as that of the adrenal and gonads. Consideration should be given to developing technics using principles of automation and telemetry for evaluation of adrenal and gonad function during and after space flights.

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